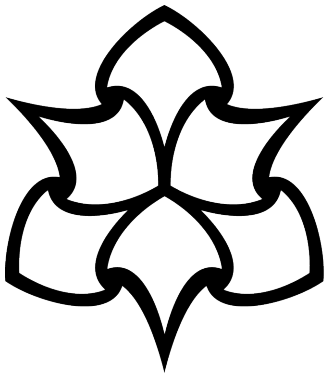

Paw Morphology in the Domestic Guinea Pig (*Cavia porcellus*) and Brown Rat (*Rattus norvegicus*).

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ABSTRACT

Mammals have adapted to different habitats, food types and modes of locomotion, which are reflected in a diverse range of paw morphologies. While the behaviour of rats and guinea pigs is well-defined, especially in terms of their locomotor and foraging behaviours, the anatomy of their foot pads has not yet been explored and compared. This study investigated adaptations in paw morphology in the domestic guinea pig (*Cavia porcellus*) and the brown rat (*Rattus norvegicus*). We predicted that rat paws would display adaptations associated with paw dexterity for handling prey items and climbing; whereas guinea pig paws would support mechanical pressure absorption for a herbivorous, sedentary and terrestrial lifestyle. Using histology techniques and scanning electron microscope, we show that rat paws have many small, deformable pads that are relatively spaced out to enable movement. The pads are clustered towards the anterior of the foot, which coincides with where the most force occurs during locomotion, as rats walk on their toes and towards the front of their paw. Guinea pigs had fewer and larger pads and the posterior pad of the forepaw was textured and contained cartilage, which may act to reduce friction and compression during standing and locomotion. We suggest that differences in paw morphology in rat and guinea pig are associated with loading during locomotion as well as paw mobility. Examining paw morphology and movement abilities in more species will give further insights in to the evolution of locomotor adaptations and paw dexterity in rodents.

INTRODUCTION

Fossil records suggest that true mammals originated around 178-168 million years ago (mya) during the Jurassic period (Reis *et al*., 2012). Comparative, skeletal analysis between the early mammalian fossils and extant species suggested *Eutherian* and *Metatherian* ancestors shared similar scansorial adaptations suited to arboreal locomotion and lifestyles (Luo *et al*., 2011). These adaptations allowed early mammals to be quick and agile, enabling them to grasp and walk over branches of varying strengths and thicknesses while foraging for live prey items, yet still retaining the ability to move over ground (Luo *et al.*, 2003). Following the Cretaceous-Paleogene mass extinction event around 66 mya, *Eutherian* and *Metatherian* ancestors thrived and diversified, becoming the origin for all modern mammalian orders (Yu *et al*., 2012). During this period of diversification, a high level of niche availability allowed arboreal mammals to radiate and adapt to aerial, aquatic and terrestrial lifestyles, leading to numerous modes of locomotion (Luo and Wible, 2005), and opening up a variety of foraging habitats. Adaptations have modified the skeletal architecture and physiology of mammalian feet, creating a plethora of foot morphologies (Lewis, 1983) that are associated with a species' habitat, locomotion style and food manipulation techniques (Toussaint *et al*., 2013). It is difficult to separate foot adaptations in relation to locomotion and food manipulation, as these are both often related to dexterity and mobility (Toussaint *et al*., 2013).

Similar environmental pressures often cause convergent adaptations of the feet and hands. Indeed, adaptations are very similar between climbing primates and marsupials, including grasping feet and reduced claws (Cartmill, 1974). However, other species, such as rodents, reveal a range of paw adaptations. For example, climbing mammals may have short claws that do not

impede grip, long curved claws for hanging, large pads to aid traction, small pads for mobility (Haffner, 1996) and fluid secretions to increase surface adhesion (Haffner, 1996; Stumpf and Welsch, 2002; Stumpf *et al.*, 2004). Additionally, skeletal modifications aid in dextrous movements and climbing. These include mobile joints that allow for directional alterations and elongated strong, dexterous digits (Haffner, 1996). Contrastingly, terrestrial mammal foot physiologies are most influenced by gravity, resulting in weight-bearing adaptations that aid the absorption and dispersal of mechanical pressures via large cushioned foot pads, subcutaneous fat pads and dense structural bones (Weissengruber *et al*., 2006; Cifelli, 1981).

While foot morphology studies have often focused on primates (Cartmill, 1974; Hamrick, 1998; Soligo and Muller, 1999; Hanna and Schmitt, 2011; Toussaint *et al*., 2013), mammals in the order Rodentia also show great diversity in their locomotor (Samuels and Van Valkenburgh, 2008), and feeding habits (Whishaw *et al*., 1998), including terrestrial, semiaquatic, arboreal, fossorial, ricochetal and gliding species from multiple families (Samuels and Van Valkenburgh, 2008). As paw morphology will be strongly associated with habitat, locomotion style and food manipulation techniques, rodents are therefore a good group from which to explore this further.

Domestic guinea pigs (*Cavia porcellus*) are diurnal, fossorial, grazing herbivores that inhabit abandoned burrows within grassy plains (Cassini and Galante, 1992). Grazing behaviour leads to a sedentary lifestyle, causing *C. porcellus* to maintain stances for lengthy periods, facilitating mastication and digestion, placing pressure on the feet and foot pads. They are quick over short distances, never venturing far from their burrows to escape predation (Cassini, 1991). Guinea pigs, and other hystricomorphs, diverged early from the murid rodents and are sometimes thought of as a separate order from Rodentia (Graur *et al*., 1991). They represent an early divergence in *Eutherian* evolution and as such often have rather anomalous characteristics

compared to other mammals, such as their facial bone structure (Muchlinski, 2008), and body muscles (Potter *et al*., 1957). Therefore, they are likely to reveal quite different foot pad adaptations compared to a murid rodent. Indeed, many murid rodents, such as the Brown Rat (*R. norvegicus*), are behaviourally flexible, capable of inhabiting numerous habitats and surviving off diverse diets (Roche and Timberlake, 1998). The dextrous paws of *R. norvegicus* allow them to climb, walk, run and jump to survive and locate resources (Roche and Timberlake, 1998), as well as precisely manipulate food items (Whishaw *et al*., 1998).

While the behaviour of rats and guinea pigs is well categorized, especially in terms of their locomotor and foraging behaviours, the anatomy of their foot pads has never been explored. The differences in their paw dexterities and locomotion styles are likely to reveal quite different paw adaptations in the two species. In particular, we described their paw morphology and texture, and also observed paw-use during forward locomotion. We would expect the rat to have long feet and digits, displaying adaptations to allow for suitable paw movements. They may also have reduced claws, as longer claws are thought to inhibit paw movements (Cartmill, 1974). Guinea pig paw are likely to have large pads containing cushioning structures to protect from mechanical pressures that arise during a non-motile, terrestrial lifestyle.

MATERIALS AND METHODS

Animals

Nine adult laboratory specimens of Wistar rat (three females and six males) and six Dunkin-Hartley guinea pigs (three females and three males) were utilized in the anatomy section of this study. Animals were size-matched to allow for comparison, and each weighed between 350 and

400g. Rats were supplied from Charles River and guinea pigs from Envigo. All animals were housed at the Biological Services Facility at University of Manchester with food and water *ad libitum*. All work in this study conformed to UK Home Office Regulations and was approved by the local ethics committee at Manchester Metropolitan University. The rats and guinea pigs were euthanized via anaesthetic overdose prior to dissection and processing. Two individuals of each species had their paws photographed against a ruler (for calibration). The area of each footpad (in black in Figure 1) and total paw area (red dotted dissection line in Figure 1) was calculated by manually tracing around these areas in Imagej (Schneider *et al.,* 2012) to give a mean pad size (area in mm) and the total percentage area of pads within the paw. All animals had the skin surface of their left fore and rear paw pad removed (see the red dissection line in Figure 1 that also denotes the total paw area) for histology. Two rats and guinea pigs also had their right fore and rear paw removed (same red dissection line in Figure 1, also denoting total paw area) for Scanning Electron Microscope imaging. Toe pads were not processed as they were too small and often separated from the rest of the paw during dissection or slicing. Samples were then flattened with closed cell foam in a histology cassette, and put in 4% paraformaldehyde (PFA) solution for 1 - 2 hours.

Staining and Imaging

Samples were loaded into the Tissue Processor (Shandon Citadel 2000) and processed in a twelve step procedure (21 hours total), which moved samples through a graded series of Industrial Methylated Spirit (IMS) bath solutions (70%, 80%, 90% and 100%), followed by Xylene immersion and paraffin wax infiltration. The samples were then embedded into paraffin wax blocks and left to set on a cold station platform at -2^{o}C for 1 hour. Each sample was sliced

in the transverse plane (see the black dotted line in figure 1c, d) with a rotary microtome (Thermo Scientific Microm HM355S) at 20µm with a 37°C water bath, then mounted (Superfrost Plus Slides) and left in an oven at 35°C to dry overnight.

Slides were stained using a Masson's Trichrome protocol developed in Grant *et al*. (2017). The slides were placed into a bath of 4% PFA for 1 hour, followed by a bath of Bouin's solution for 3 hours. The samples were first cleared via xylene baths, followed by re-hydration using descending IMS solutions (100%, 90%, 70%) in preparation for staining. Masson's Trichrome Staining involves several solutions: Biebrich Scarlet Acid, Acid Solution (25% Phototungstic Acid, 25% Phosphomolybdic Acid and 50% double distilled water (ddH_2O)), Aniline Blue and Acidified Water, with numerous ddH_2O washes throughout. After staining, the tissue slices were dehydrated using the reverse of the rehydration process (IMS 70%, 90% and 100%) followed by xylene, towel dried, coated in DPX mounting medium, cover-slipped and left to dry. Once dry, excess mounting medium was removed from the sides and surface of the coverslip using a razor blade in preparation for microscope imaging.

An Axio Imager M1 Brightfield microscope and AxioCam HRc (Carl Zeiss) camera were utilized to image the tissue slices using the Zeiss ZEN Pro imaging software. These tools allowed for adjustments in white balance, exposure and magnification (x20 - x150), enabling the collection of visual, photographic data for each slide and tissue sample at varying magnifications.

Scanning Electron Microscopy (S.E.M.)

Individual rat and guinea pig foot pad samples were removed from the 4% PFA and placed into a Sputter Coater to cover the foot pad samples in a three-nanometre thick layer of conductive gold

to allow for subsequent S.E.M. scanning. Each foot pad sample was placed into the S.E.M. scanner and images were taken at 10µm at each labelled pad location (Figure 2) to examine the pad texture.

Gait Analysis

Three adult female rats and four adult female guinea pigs were used in the behavioural section of this study. Three domestic guinea pigs were filmed in Williamson Park Zoo, Lancaster and one in Heeley City Farm, Sheffield. Three brown rats were filmed at the Wildwood Trust, Kent. The animals were all kept inside and had food *ad libitum*. The guinea pigs were kept under natural light, whereas the rats were kept under a reversed light schedule, therefore both species were tested in the "awake" part of their day. Rats and guinea pigs were filmed, in the day, using a custom behavioural filming set-up (see Grant *et al*., 2018 for specific details). Individual animals were placed into a Perspex arena with pedobarograph floor. 1.5 second recordings were collected when the animal locomoted forward. A glass pedobarograph floor highlighted the position of paw contacts and distribution of contact over the foot. A high-speed video camera (Phantom Miro ex2) recorded from below the set-up at 500 frames per second. A measure of pad deformation was processed from one video clip from each of the individuals, for both one fore and one rear paw. The pedobarograph highlighted which area of the foot was contacting the floor. Therefore, the contacting area was approximated by drawing around the contacting image from the footage in ImageJ (Schneider *et al.*, 2012) during stance phase, and 30 ms before the stance phase - as the pads were all contacting the floor but force was still being applied. The change in contacting area between these two frames was calculated as a percentage change.

RESULTS

Paw Morphology and Texture

Rat forepaws contained four digits (Figure 1a) and their rear paws contained five digits (Figure 1e). The animals were chosen to be size-matched, therefore, their feet were similar in length. Rat forepaw length was 1.8-2.0 cm and rear paw length was 3.5-3.7 cm, forepaw width was 0.9-1.0 cm and rear paw width was 1.0-1.1 cm (Figure 1). The shape of the rats' rear feet gave the paws a slimmer appearance, as they had long toes and the heel was tapered, giving it a slim profile (compare Figure 1e and f). Indeed, the toes were long in rat rear paws, contributing to 24.7% of the total paw length (observe the longer rat toes in figure 1e, compared to 1f). Rats also had thin claws that were relatively flexible (note the damage to the rear claw in Figure 1e). The pads of the feet varied between the species, with rats having smaller, more numerous pads than the guinea pig (Figure 1). Rat forepaws contained five pads. Three pads were located within the anterior section (Figure 1a, labelled a1-3) and two pads were located within the posterior section (Figure 1a, labelled p1-2). The rear paw contained six pads. Five pads were located within the anterior section (Figure 1e, labelled a1-5), and one in the posterior section of the pad (Figure 1e, labelled p1). Each pad was spaced out, with clear gaps between them (Figure 1a, e). In lateral view, the rat pad did not have a clear arch between the anterior and posterior section (Figure 1c), causing the foot to lie flat against a surface; apart from the toes, which were relatively curved and did not contact the surface in their posterior sections. Each of the forepaw pads had a mean size (area) of 0.59±0.04 mm, and the total pads occupied 30.03% of the total forepaw area. Each

of the rear paw pads had a mean size (area) of 0.61±0.05mm, and the total pads occupied 17.35% of the total rear paw area. Rat footpads had shallow irregular surface textures (Figure 2, top panels (2a-g)). The most anterior pad (Figure 2a) and the anterior, lateral pad (Figure 2b) of the forepaw also displayed a texture consisting of overlaid skin layers with a scale-like appearance. In rat, footpads on the forepaw appeared to have more variability in texture than in the rear paw; forepaws contained both scale textures (Figure 2b) and irregular textures (2a, c-d), whereas rear paws only contained irregular textures (Figure 2e-g).

Guinea pig forepaws also contained four digits (Figure 1b), but their rear paws contained only three digits (Figure 1f). Guinea pig paw length was similar to rat (forepaw length was 2.0-2.5 cm and rear paw length was 3.5-5.6 cm), but paw width was slightly wider (forepaw width 1.1-1.4 cm and rear paw width 1.1-1.3 cm) (Figure 1). Guinea pig toes were shorter than rat, contributing to 20.4% of the total paw length, and their claws were thick and stiff (Figure 1b and f). Guinea pig forepaws contained four pads. Three pads were located within the anterior section of the paw (Figure 1b, labelled a1-3), with the largest pad positioned central and anterior to the two peripheral smaller pads (labelled a2 in Figure 1b). There was also one single, large pad in the posterior section (Figure 1b, labelled p1). The rear paw contained four pads. There were two pads within the anterior section of the rear paw (Figure 1f, labelled a1-2) and two larger pads within the posterior section of the paw (Figure 1f, labelled p1-2), one of which was elongated, covering almost the entirety of the lateral periphery of the rear paw (p2 in Figure 1f). Each of the forepaw pads had a mean size (area) of 1.77±0.12mm and the total pads occupied 57.13% of the total forepaw area. Each of the rear paw pads had a mean size (area) of 2.14±0.15mm and the total pads occupied 29.69% of the total rear paw area. This indicated that the guinea pig footpads were larger and closer together than the rat (compare Figure 1a, e to Figure 1b, f; and observe

diagrams in Figure 2), although they appeared slimmer in profile (depth) (Figure 1c and d). The largest spacing between the pads in the fore and rear paws in guinea pig occurred between the posterior and anterior sections, which were divided by a defined arch. This arch also caused the paw to not lie flat against a surface, only contacting at the posterior and anterior pads (Figure 1d). Guinea pig pads had an irregularly patterned surface texture across all pads (Figure 2h-k).

Paw Epidermis

The paw epidermis was examined in superficial slices (those which are closer to the surface of the pad). Both guinea pig and rat paws contained distinctive epidermal pegs, these can be seen as side-on projections (EP in Figure 3a, b, c, e), and can also appear in superficial slices as cross-sections of the epidermal pegs projecting into the dermis (EPc in Figure 3a and b), or cross-sections of the dermal papillae projecting in to the epidermis (DPc, Figure 3d and f). Epidermal pegs were observed in the fore and rear paws of both rat and guinea pig. The epidermal pegs of the rat had depths of 0.27±0.05 mm (Figure 3a). The epidermal pegs of the guinea pig appeared shallower, with depths of 0.17±0.04 mm (Figure 3e), and were less regular than that of the rat.

Paw Internal Structures

Collagen fibers were stained blue in the dermis in both rat and guinea pig (Figure 3, Figure 4) and muscles were stained red (Figure 4). Muscle fibers were seen in all the paws in both rat and guinea pig (M in Figure 4a-d). This is skeletal muscle with clear striations observed in Figure 4c and 5c. Attachment points were difficult to identify due to the removal of the paw skin from the bones, and will be considered more in the discussion. Adipose (fat) tissue can also be seen in both rat and guinea pig paws, although it appeared to be more prevalent in guinea pig paws (F in

Figure 4a, c and d). The rear paws in rat revealed muscles, which ran posteriorly-anteriorly along the pad (Figure 4b). Muscle attachment points were not visible in many of our slices, although a possible attachment of a muscle to a tendon is visible in the rear paw example in Figure 4b (indicated with an asterisk).

In the guinea pig, there were fewer muscle fibers oriented parallel to the plane of section, therefore most muscle fibers appeared as cross sections in both fore and rear paws (Figure 4c, d). Attachment points could only be observed in the forepaws in our slices (indicated by an asterisk), beneath the anterior pad in Figure 4c and the anterior pad in Figure 5c. In the guinea pig, the deep slices of the posterior pad of the forepaw contained a structure (Figure 5), consisting of hyaline cartilage, with clear chondrocytes (C in Figure 5b, inset). This cartilaginous structure was concentrated within the center of the posterior pad in Figure 5a, but appeared next to the epidermis in deeper slices (Figure 5b and c), indicating its dome-shape. In dissection, the cartilaginous structure could be seen deep within the center and posterior of the posterior pad (Figure 5a).

Gait Analysis

Rats walked by putting their toes and anterior part of the paw down first on to the floor, and then the heel of the pad (Figure 6g). This sequence also occurred in the rear paw, and was reversed during lift off. During stance phase, with the foot flat on the floor, the rat exerted pressure on to the toes and paw, with the most amount on the anterior pads (Figure 6a). As the rat placed more weight on the three anterior paw pads over the sequence in Figure 6g, they deformed and spread together. Indeed, between panel 3 and 6 in Figure 6g, the three pads increased in total area by 61.0%.

The guinea pig put the posterior and middle of the paw down first on to the floor, and then the heel and toes (Figure 6h). This sequence also occurred in the rear paw, and was reversed during lift off. During stance phase, the guinea pig exerted pressure mainly on the paw, particularly the posterior pad (Figure 6b). Between panel 3 and 6 in Figure 6h the posterior pad only increased by 6.9%. Indeed, rat fore (38.5±23.3 %) and rear paws (31.3±28.6) (Figure 6e) deformed, on average, more than guinea pig fore (8.3±4.7 %) and rear paws (15.3±15.7 %) (Figure 6e-f). This pattern was found across all the tested animals.

DISCUSSION

Foot pad morphology differed greatly between the study specimens, especially in the forepaws. Rat feet had long digits and numerous, spaced-out, deformable footpads, which were likely to not inhibit their precise paw control and movements. Guinea pigs had fatter digits and fewer, larger pads. They also had a cartilaginous structure in their forepaw heel.

Rats have dextrous paws in order to grip objects and to climb and manouver around their environments (Karantanis *et al*., 2017). Rat fore and rear paws have numerous, small pads, that were spaced out (Figures 1 and 2). This arrangement allows the paw to easily move around and especially to close tightly. This enables precision gripping, and the handling and grasping of narrow objects and substrates. Elongated digits, combined with a greater number of digits of the rear paw, also provide rats with the ability to grip objects, and has been associated with a scansorial lifestyle and dextrous paws in marsupials, rodents and primates (Cartmill, 1974). Indeed, previous studies, have found that climbing rodents have large joint mobility as well as elongated, slim limbs (Haffner, 1996; Thorington, 1972; Thorington and Heaney, 1981; Samuels

and Van Valkenburgh, 2008). Although rats have been observed to dig and maintain burrows, their reduced claw size indicates that they may be more adapted for a scansorial lifestyle, since large claws are thought to reduce paw and digit mobility (Cartmill, 1974), and being entirely clawless is associated with arborealism in primates (Toussaint *et al*., 2013).

Guinea pigs are terrestrial and vegetarian; they do not manipulate objects with their paws and therefore make less skilled forepaw movements than rats (Whishaw *et al*., 1998). Guinea pig fore and rear paws have few, closely-spaced, large pads (Figures 1 and 2). These pads create large contact areas with the ground to spread and distribute ground forces (Cassini and Galante, 1992). Previous studies have suggested that the sedentary, terrestrial lifestyle of guinea pigs have resulted in high pressures being placed onto the paws and pads (Uchida *et al.*, 1988). The larger, thicker claws and digits of the guinea pig could also be an adaptation to their fossorial lifestyle, being used to maintain or improve burrows (Woodman and Gaffney, 2014). Having fewer, large foot pads is also associated with being fossorial as they form a relatively flat surface, which allows soil to move smoothly over the foot, which has been observed in *Arvicola terrestris* and *Heterocephalus glaber* (Haffner 1998).

We also observed a hyaline cartilaginous structure (Figure 5) within the posterior forepaw pad in guinea pigs (Figure 2i and C_H in Figure 5b and c). Cartilage is well-adapted to complex loading patterns and is resistant to compression (Franke *et al*., 2007). Indeed, we saw that the guinea pig forepaw pad contacting area in Figure 6h changed by only 7% during the locomotion sequence, indicating that it did not deform much against the floor. Cartilage can be found around the feet of rodents (Haffner, 1998), elephants (Hutchinson *et al*., 2011), and horses (Bowker *et al*., 1998). However, the placement of cartilage can vary greatly between species, even in small quadrupedal rodents such as *M. avellanarius, M. minutus, M.* domesticus and *A.*

terrestris. We suggest that the position of this structure beneath the posterior forepaw pad is likely to reduce the forces that occur during terrestrial locomotion and standing in guinea pigs, since cartilage is resistant to compression. When the guinea pig foot was in stance phase, forces were focused on the posterior pads (Figure 6b). The large posterior pad coupled with the defined arch of the foot in guinea pigs (Figure 1d) pushes the foot back and focusses forces on to the pad containing the cartilaginous structure. We even observed that some of the guinea pigs in our gait studies only had the posterior pad contact the floor, with none of their toes in contact. Indeed, only light toe contact can be seen in Figure 6b and d. This agrees with work by Santangelo *et al*., (2014) and Blair (2013), who also identified that pressure concentrated in the middle and anterior pads in guinea pigs during walking.

Rat pads lacked this cartilaginous structure. Rat pads were numerous, larger in depth than guinea pigs (compare pads in Figure 1c and d), and deformed more against a surface (Figure 6g). This deformation of the pad is likely to increase the coefficient of friction and grip during climbing (Cartmill, 1974). The deformation of rat pads against a surface can be observed in the gait images in Figure 6g, where the pads increased and spread together (with a 61% increase in pad area) as more force was applied during locomotion. Rats walk primarily on the tips of their digits and anterior pads, placing little pressure on their heels (Figure 6a, c), which is also corroborated by Schmidt and Fischer (2010), Batka *et al*. (2014) and Mendes *et al*. (2015). Rats have more pads in the anterior section of their paws, which may act to reduce pressure during this type of locomotion. They also have a thick epidermis, with clear epidermal pegs, which are thought to protect from mechanical strain (Haffner 1998; Bove *et al*., 2016; Miao *et al*., 2017), (EP in Figure 3a, b and c). The thick epidermis is also associated with an increase in the number

of glands which produce fluid to increase surface friction during climbing (Haffner 1998), although we did not observe any fluid secreting glands in our preparation.

Both species had relatively smooth pads, which lacked any defined skin ridges that can be observed in other climbing animals, such as in *Otolemur crassicaudatus* (see Figure 1 in Cartmill, 1974). There were some irregular surface textures (Figure 2), particularly in the areas that contact surfaces the most, perhaps being involved in increasing the coefficient of friction with a surface. Pad texture was similar across the guinea pig foot pads (Figure 2). However, in rat, the anterior, lateral pads in rat forepaws had a scale-like texture (Figure 2a,b), which also coincides with their gait and stance being more focussed towards the front of the foot. Finding this texture on the lateral (outside) pads in rat is harder to explain, but perhaps is useful during gripping on to a vertical surface when the feet are oriented outwards, or holding objects, where gravity will exert force downwards in this orientation towards the lateral pads.

While many differences were observed in pad arrangement and structure, the anatomy of the foot was relatively similar between both species. All paws contained an epidermis, with skeletal muscle and fat within the dermis. It was difficult to see where the muscles attached in most cases, due to dissecting them away from the bone, and also the orientation of our slices. However, example rat muscle fibers attached to a tendon (Figure 4b), and were probably associated with contraction and movement of the toes (Hildebrand, 1978). The guinea pig muscle example appeared to attach beneath the anterior and posterior pads of the fore foot (Figure 4c and 5c). Contraction of this muscle is likely to pull the pads together for gripping (Hildebrand, 1978). Insertion of flexor muscles within plantar pads have previously been observed in climbing, digging, swimming and generalist rodents (Hildebrand, 1978). Paw muscles and their attachment positions vary greatly between different species of rodents (Rocha-Barbosa, 2007;

Hildebrand, 1978). However, it is not just muscles, but many other structures (including bone, cartilage, tendons and ligaments) that all act to constrain and shape paw movement in response to muscle activation (Hildebrand, 1978). Indeed, it is challenging to infer paw movement capabilities from studying foot anatomy in isolation, and gait measures are a useful addition to anatomical studies of this nature.

While forelimbs and hindlimbs are both used in climbing and quadrupedal walking (Samuels and Van Valkenburgh, 2008), we observed more specific adaptations in the forepaw in both animals. In the forepaws, guinea pigs had cartilage within the posterior pad (Figure 5), and rats had more deformable pads (Figure 6). Forepaws are used in the gripping of surfaces during climbing and in the manipulation and handling of objects, such as prey items. Whishaw *et al*., (1998) suggested that the terrestrial lifestyle of the guinea pig, coupled with its vegetarian diet, meant that it manipulates objects less and therefore has less skilled forepaw movements. They went on to suggest that skilled forelimb movements may have been part of the repertoire of a common ancestor for rats and guinea pigs, but is not present in guinea pigs. Reduction in the capacity to move the forepaw is complimented by the appearance of large cushioned pads in the guinea pig, as these do not promote the ability to move the paw.

Examining paw morphology and movement abilities in more species will give further insights in to the evolution of locomotor adaptations and paw dexterity in rodents. However, applying these observations to a broader range of species is likely to be challenging as locomotion modes and object manipulation strategies vary considerably between species (Toussaint *et al*., 2013), and paw morphologies are also diverse (Cartmill, 1974). Nevertheless, exploring paw morphology and anatomy over a range of species will give important insights in to the evolution of food acquisition and locomotion.

CONCLUSIONS

Here, we made simple descriptions of the paws of guinea pig and rats and considered our findings in terms of locomotion, as well as their ability to move during object manipulation. We found that rats had small, spaced, deformable pads, which are likely to allow for the range of paw movements that rats make during object manipulation and complex locomotion, such as climbing. Rat pads were focussed towards the anterior section of the paw and their anterior pads were also more textured, which reflects how they locomote on their toes and front pads. Guinea pigs had large, flat pads, with the posterior forepaw pad containing cartilage, which resists compression. These structures are likely to be associated with sedentary standing in guinea pigs on the posterior area of their feet.

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FIGURE CAPTIONS

Figure 1. Pad morphology of rat and guinea pig. Rat fore (a) and rear paw (e) photographed in palmar view. Rat (c) and guinea pig (d) forepaws in lateral view, the dashed black line indicates the orientation of transverse sections for histology. Guinea pig fore (b) and rear (f) paw photographed in palmar view. In panels a, b, e and f: the dotted red line highlights the area dissected for histology, the black circles indicate the individual footpads, and the thicker black line indicates the foot arch in guinea pigs (in panels b, d and f only). Pads are numbered and referred to as ‘a’ for anterior pads, and ‘p’ for posterior pads. Scale bars are 5mm.

Figure 2. Rat (top) and guinea pig (bottom) paw morphologies and textures, with forepaws (on the left) and rear paws (on the right). Panels a-k reveal 10µm S.E.M images of the surface texture of each correspondingly lettered, individual footpad in the palmar diagram. Red dotted line denotes area dissected.

Figure 3. Rat (top) and guinea pig (bottom) forepaw (left) and rear paw (right) superficial slices. Masson's trichrome staining of transverse slices, showing epidermis in red and dermis in blue. The epidermal pegs (EP) are visible from the epidermis into the dermis and can be seen in cross section (EP_C) in superficial slices of the dermis. Cross sections of the dermal papillae can also be seen in superficial slices of the epidermis (DP_C). Palmar paw diagrams contain a red dotted line that denotes the total area dissected, red shading indicates the position of the panel, also denoted by the letter (a-f). Examples are from three different rat individuals and one different guinea pig. Scale bars are 1 mm.

Figure 4. Internal structures within the forepaw (left) and rear paw (right) of rat (top) and guinea pig (bottom). Muscles (M), in red, were seen in the deeper layers of the pads in rat (a, b) and guinea pig (c, d). Fat (F) can also be seen in forepaw of rat (a), and throughout the fore (c) and rear paws (d) in guinea pig. Asterisks in panel b and c correspond to likely muscle attachment points. Palmar paw diagrams contain a red dotted line that denotes the total area dissected, red shading indicates the position of the panel, also denoted by the letter (a-d). Examples are from one different rat individual and two different guinea pigs. Scale bars are 1 mm.

Figure 5. Internal cartilage within guinea pig forepaw. The posterior pad of the guinea pig forepaw contained a large dome of hyaline cartilage (C_H) (shown in during dissection (a) and in slice (b, with magnified inset on left, and c). The cartilage contained chondrocytes (C) (in red in inset panel, and shown throughout in b and c). Asterisks in panel c correspond to likely muscle attachment points. Palmar paw diagram contains a red dotted line that denotes the total area

dissected, red shading indicates the position of the imaged panel. Examples are from two different guinea pig individuals. Scale bars are 1 mm. M: muscles, F: fat, EP: Epidermal pegs

Figure 6. Example force distribution during locomotion in rat and guinea pig during walking. Forepaw stance phase in rat (a) and guinea pig (b); rear paw stance phase in rat (c) and guinea pig (d). The % total pad area (mean with standard error bars) increased during locomotion in both species, but more so in rat (e) compared to guinea pig (f). Example forepaw locomotion in rat (g) and guinea pig (h). Rats put the toes and anterior section of the paw down first (panel 1), and then the heel (panel 6). Guinea pigs put the center of their paw down (panel 1) and then the heel and toes (panel 6). The sequences are over 60 ms taken every 10 ms. A colour map version of panel 6, with the foot flat on the floor (stance phase), can also be seen for both rat (a) and guinea pig (b).